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Development of a Soil Bioassay for Triclopyr Residues and Comparison with a Laboratory Extraction

R. D. Ranft, S. S. Seefeldt, M. Zhang, and D. L. Barnes*

The use of triclopyr for the removal of woody and broad-leaf vegetation in right-of-ways and agricultural settings has been proposed for Alaska. Triclopyr concentrations in soil after application are of concern because residual herbicide may affect growth of subsequent vegetation. In order to measure triclopyr residues in soil and determine the amount of herbicide taken up by the plant, soil bioassays were developed. Four agricultural species, turnip, lettuce, mustard, and radish, were tested to determine sensitivity to triclopyr in a 1-wk bioassay. The sensitivity (I_{50}) of turnip, lettuce, mustard, and radish was 0.33 ± 0.05 kg ai ha^{-1}, 0.78 ± 0.11 kg ai ha^{-1}, 0.78 ± 0.07 kg ai ha^{-1}, and 0.85 ± 0.10 kg ai ha^{-1} (mean ± SE), respectively. Mustard was the most consistent crop in the bioassay with a midrange response to triclopyr and lowest standard deviation for germination as compared to the other species. Thus, it was used in a bioassay to determine triclopyr concentrations in a field trial. The bioassay of mustard closely matched residual amounts of triclopyr in a field trial determined by chemical extraction. Estimates of residual triclopyr concentrations using the bioassay method were sometimes less than the triclopyr concentration determined using a chemical extraction. These differences in concentrations were most evident after spring thaw when the chemical extraction determined there was enough triclopyr in the soil to reduce mustard growth over 60%, yet the bioassay measured only a 10% reduction. The chemical extraction method may have identified nonphototoxic metabolites of triclopyr to be the herbicidal triclopyr acid. These methods, when analyzed together with a dose–response curve, offer a more complete picture of triclopyr residues and the potential for carryover injury to other plant species.
Nomenclature: Triclopyr; lettuce, *Lactuca sativa* L.; mustard, *Brassica juncea* (L.) Czern.; radish, *Raphanus sativus* L.; turnip, *Brassica campestris* L.
Key words: bioassay, triclopyr, dose response.

El uso de triclopyr para la eliminación de la vegetación arbórea y maleza de hoja ancha de la manera correcta y en sitios agrícolas ha sido propuesto para Alaska. Las concentraciones de Triclopyr en el suelo después de la aplicación son motivo de preocupación debido a que sus residuos podrían afectar el crecimiento de la vegetación sub-secuente. Para poder medir los residuos de triclopyr en el suelo y determinar la cantidad de herbicida absorbido por la planta, se desarrollaron bioensayos de suelo. Cuatro especies agrícolas: nabo, lechuga, mostaza y rábano fueron probados para determinar la sensibilidad al triclopyr a una semana del bioensayo. La sensibilidad del nabo, lechuga, mostaza y rábano fueron I_{50}'s 0.33 Kg ± 0.05, 0.78 ± 0.11, 0.78 ± 0.07, 0.85 ± 0.10 SE kg ia/ha respectivamente. La mostaza fue el cultivo más consistente en el bioensayo con una respuesta a media dosis de ctriclopyr y la más baja desviación estándar para la germinación cuando fue comparada a otras especies. Por lo tanto se usó en un bioensayo para determinar la concentración de triclopyr en un estudio de campo. El bioensayo de la mostaza cercanamente coincidió con las cantidades residuales de triclopyr por análisis químico, en un estudio de campo determinado por extracción química. Las estimaciones de las concentraciones residuales de triclopyr usando el método de bioensayo, fueron algunas veces menores que la concentración de triclopyr determinada cuando se usa una extracción química. Estas diferencias en concentraciones fueron más evidentes después del deshielo de la primavera cuando la extracción química determinó que había suficiente triclopyr en el suelo para reducir el crecimiento de la mostaza en más del 60%, aunque el bioensayo registró solamente una reducción del 10%. El método de extracción química pudo haber identificado metabolismos no fototóxicos de triclopyr por ser un herbicida de triclopyr ácido. Estos métodos, cuando se analizaron junto con una curva de la respuesta a diferentes dosis proporcionaron una idea más completa de los residuos de triclopyr y del potencial de daño posterior a otras especies de plantas.

Triclopyr is a selective herbicide used to kill unwanted broadleaf plants (Cox 2000). Triclopyr has been used in some agricultural settings, but its current usage is mainly for controlling woody plants and broad-leaved weeds in utility and roadside right-of-ways, for conifer release, and on site preparation in forestry (Lee et al. 1986; Nelson et al. 2006). Triclopyr is currently being studied to determine the effect on the woody and broad-leaf vegetation of the Conservation Reserve Program (CRP) land near Delta Junction, AK (Ranft 2008) and roadside right-of-ways (Rhodes 2008). Control of woody vegetation in CRP land is required by United States Department of Agriculture (USDA) CRP regulations to keep fields in a condition such that the land can be easily returned to crop production (United States Department of Agriculture 1986). Triclopyr residues in the soil are of concern due to the effects they may have on subsequent germination of plant species (Norris et al. 1987). Over 50 years ago it was shown that herbicide persistence can have a drastic effect on the yield of an agricultural crop if planting is too soon after herbicide application (Weaver 1948).

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Herbicide persistence in soil can vary among soils and climates. The reported half-life of triclopyr ranges from 10 to 100 d and was longer on forested sites than on agricultural sites (Cox 2000). The half-life of triclopyr in soil studies in Oregon, USA (application rate of 10.1 kg ai ha^{-1}) and Ontario, Canada (application rate of 3 kg ai ha^{-1}) were 75 and 69 d, respectively (Norris et al. 1987, Thompson et al. 2000). Results of a high latitude triclopyr study in Sweden (application rate of 1.9 kg ai ha^{-1}) measured a persistence time in soil from 1 yr to more than 2 yr (Torstenssen and Stark, 1982). Our study was located in interior Alaska at high latitude, not unlike that of Sweden. Thus, attention was given to the possibility of increased herbicide persistence in the study site due to previous findings at high latitudes and climate conditions that can keep soils frozen for 6 to 7 months of the year (Knight and Lewis 1986).

With a chemical extraction method, changes in herbicide concentration in soil over time can be determined and a half-life calculated, assuming the results follow first-order decay. A chemical extraction is often used to determine the amount of herbicide in the soil, both available and unavailable to the plants (Hamaker and Goring 1976). Because it is not possible to differentiate between available and unavailable herbicide, a chemical extraction will give no indication of potential plant response (Eberle and Gerber 1976).

As an alternative to chemical extraction methods, some researchers use plants as bioindicators (i.e., bioassay) of toxicants in soil (Wang and Freemark 1995). A bioassay is a measure of a plant's response to the total herbicide residue in soil at a site-specific location (Ferris and Haigh 1992). A bioassay can determine whether or not a susceptible crop grown in a rotation will be damaged, even when current recommended recropping intervals are followed (Jettner et al. 1999). For example, mustard was found to be a good indicator of 2,4-D (Mitchell and Marth 1946), and ryegrass (*Lolium multiflorum* Lam.) was chosen as the best indicator among several crops for linuron (Dubey and Freeman 1963). A bioassay on rotational crop response of the herbicide propoxycarbazone used for controlling broadleaf weeds in wheat fields in the Pacific Northwest, determined that planting too soon after application reduced some crop yields by 50% (Rainbolt et al. 2001). The advantage of using a bioassay is its simplicity and repeatability. Bioassays are an indirect measurement of soil herbicide concentration, but a direct measure of plant available herbicide (Eberle and Gerber 1976).

In order to determine triclopyr concentration in Alaska soils, a laboratory bioassay using turnip, lettuce, mustard, and radish and a range of triclopyr concentrations was implemented. The objectives of this study were to develop a soil bioassay to determine the response of these plants to triclopyr; compare the dose–response of mustard to triclopyr in a 1-wk bioassay; and compare the herbicide concentration measured in a field study using a plant bioassay to a chemical extraction.

Materials and Methods

Growth Response to Triclopyr in Soil One Week after Treatment. To determine growth response to soil-applied triclopyr, soil from Delta Junction, AK (coarse-loamy, mixed, superactive, nonacid Typic Cryofluvents) (Pink 2008) was collected with a shovel, at random locations, in one field, to a depth of 10 cm, from a post-tilled agricultural field and placed in a 53-L plastic container with lid. The soil (pH of 4.7) was stored at room temperature for 3 d before use. Plastic trays[1] (3 × 25 × 25 cm) were filled with 1,250 ± 0.5 g of soil. Calculated on an area basis, triclopyr[2] was applied to soil at rates of 2.2, 1.1, 0.55, 0.275, 0.138, 0.069, and 0.0 kg ai ha^{-1}, with three replications. At the time of application 10 mL of herbicide solution was sprinkled over the soil in the trays and mixed by hand for 1 min. The tray was divided into four equal sections (156 cm^2), with 20 seeds[3] of each species sown into a section. A thin layer of untreated soil (~ 2 mm) was added to cover the seeds. Water was then added to bring soil to field capacity and each tray was covered with a clear plastic cover to help keep the soil moist. Trays were placed at random on a laboratory bench and the soil was watered daily. The trays were randomly moved each time they were watered to minimize the impact of microenvironment on germination. The laboratory was kept at 20 C, with a 12/12 hr day/night photoperiod. After 1 wk, germinated plants were clipped at the soil surface, dried (65 C for 48 hr), and weighed. This trial was conducted two additional times resulting in nine replications of each herbicide treatment.

Determination of Triclopyr Concentration in the Field. Field experiments were carried out at two farms near Delta Junction, AK, one at 63°54′30.12″N, 145°11′51.42″W, and the other at 63°59′53.04″N, 145°25′58.14″W. The soil at both sites was a coarse-loamy over sand or sandy-skeletal mixed, superactive Typic Haplocryept (Pink 2008). The soils organic carbon and nitrogen were tested using Leco, TruSpec,[4] bulk density was determined by calculating the dry weight of the soil over the volume sample, and pH was determined using a Corning pH meter.[5] Each site was covered with woody, broad leaf, and grass vegetation with a few bare soil patches.

At each site, an experiment was set up with four replications of triclopyr at 2.2 kg ai ha^{-1}. The dimensions for each plot were 2 m by 10 m, with 0.5 m of space between the plots to avoid spray overlap. On July 17, 2006, the vegetation on the experimental area was mowed to a height of 15 cm and left on the plots. Herbicide application followed a few hours afterwards. Triclopyr was applied with a CO_2 backpack sprayer[6] and a 1.8-m boom with four spray nozzles, at a height of 30 cm. The spray nozzle was an even flat-fan 8002[7] and delivered 190 L ha^{-1} at 240 kPa. Twelve 15-cm soil cores were collected randomly throughout each plot with a 2-cm diameter corer. The 12 cores in a plot were separated into 0- to 5-cm and 5- to 15-cm fractions. Each fraction in a plot was combined, placed in a ziplock bag, and stored in a cooler with ice. The soil corer was cleaned with acetone between the sampling of each plot. Soil samples were collected (avoiding previous coring holes) from each plot 1, 3, 7, 21, 35, 283, and 365 d after treatment (DAT) and kept frozen (−30 C) until they were needed for the bioassay or extraction. Control soils were collected more than 2 m away from herbicide-treated plots to prevent contamination from application or lateral movement of the herbicide. Soils used in this study were tested, and organic carbon, nitrogen, and cation exchange

Table 1. Properties of Typic Haplocryepts soil sampled near Delta Junction, AK.

Property	Depth		
	0–5 cm	5–15 cm	15–30 cm
pH	4.6 ± 0.12[a]	4.6 ± 0.12	4.6 ± 0.12
Bulk density	0.91 ± 0.28 mg/m^3	0.88 ± 0.18 mg/m^3	1.20 ± 0.39 mg/m^3
Total C	22.68% of < 2 mm	5.49% of < 2 mm	0.47% of < 2 mm
Total N	0.794% of < 2 mm	0.233% of < 2 mm	0.051% of < 2 mm
CEC[b]	44.4 mol/kg	25.2 mol/kg	15.6 mol/kg

[a] Standard error.

[b] Abbreviation: CEC, cation exchange capacity.

capacity decreased with depth of soil (Table 1). Soil bulk density increased with increasing soil depth whereas pH was constant throughout (Table 1). Temperature and precipitation data were obtained from a National Oceanic and Atmosphere Administration weather station (Delta Junction 20SE) located near the study site.

A subsample of soils collected from each plot at each sampling time was thawed at room temperature (3 to 4 hr), and thoroughly mixed by hand starting with the controls from each site and then working with herbicide applied soil. Hands were washed thoroughly between sampling times as to not contaminate soils from a previous date. Soil then was placed into two 153-cm^2 petri dishes[8] (190 ± 0.5 g/dish). Twenty mustard seeds were sown on each petri dish. Mustard was selected to be used in the field soil bioassay experiment based on the midrange response to triclopyr and germination rate in the species comparison trial. Petri dishes were placed in a growth chamber[9] with a temperature of 18 C/20 C night/day, relative humidity of 75/65% night/day, and a 12/12 hr night/day cycle. The soil was watered as needed and petri dishes were randomly rotated daily for 1 wk to compensate for micro temperature and humidity variations in the growth chamber. More than 65% of the mustard seeds germinated after the first day. One week after seeding, mustard plants were 15 to 20 mm tall in the control treatments, with their second true leaf emerging, which was similar to the sizes and leaf number obtained during the development of the bioassay. At that time all sprouted plants were cut off at the soil surface, dried at 65 C for 48 hr, and weighed.

A second subsample of soils collected from each plot at each sampling time was used for a chemical extraction of triclopyr in the laboratory. A method developed by Tsukioka et al. (1986) with modifications was used to extract triclopyr from the soil. Boron trifluoride was used in the esterification procedure instead of diazomethane which can be highly explosive, according to Mulkey (1990). The modified procedure had an average recovery of 73.6 ± 6.9% (Mulkey 1990). A centrifuge with settings of 1,509 × *g* for 1 min was used instead of the filter process in Mulkey (1990) for separating the soil and water solution as well as the ether and water solution. A turbo evaporator[10] was used in the esterification procedure to increase the evaporation rate of the ether and hexane (Ranft 2008; Rhodes 2008).

The gas chromatograph (GC) used in this study was an Agilent 6890N Network GC system with a 5973 Network Mass Selective Detector (MS).[11] The settings for the GC were temperature of 280 C, pressure 2.49 psi, 100.0 mL min^{-1} purge flow, and 36.4 min run time. The MS parameters acquisition mode was selected ion monitoring with a total of 24 ions including 210 and 212 for triclopyr, and the data analysis was sorted by retention time.

Statistics. The bioassay experiments were conducted using a randomized complete block design. All data from these two experiments were normally distributed. Each experiment was subjected to an ANOVA in SAS[12] and probability (P values) ≤ 0.05 was considered significant. No significant differences were measured among the three times in the first bioassay experiment and the two sites in the second bioassay experiment (P = 0.27) so data were combined for subsequent analyses. Nonlinear regression analysis using the log-logistic dose response model

$$y = C + (D - C)/(1 + \exp\{b[\log(x) - \log(I_{50})]\})$$

where C = lower limit, D = upper limit, I_{50} = dose giving 50% response, and b = the slope (Seefeldt et al. 1995) was used to compare the dose responses of the bioassay experiments.

Results and Discussion

Growth Response to Triclopyr in Soil One Week after Treatment. The four plant species were not equally sensitive to triclopyr (P < 0.005). Turnip was the most sensitive species to triclopyr (I_{50} = 0.33 kg ai ha^{-1} ± 0.05 SE) (I_{50} is the amount of herbicide that reduces plant biomass 50% compared to controls). Lettuce, mustard, and radish were less sensitive with I_{50}'s of 0.78 ± 0.11 kg ai ha^{-1} SE, 0.78 ± 0.07 kg ai ha^{-1} SE, and 0.85 ± 0.10 kg ai ha^{-1} (mean ± SE), respectively (Figure 1). These results highlight the differential sensitivities of plant species to triclopyr. In the development of a bioassay procedure, when there is an unknown concentration of herbicide in soil, it may be best to use plant species with a range of sensitivities to accurately determine the herbicide concentration and reduce the risk of false positives or negatives. Of the four species, mustard was selected to use in the field study due to its midrange response to triclopyr, higher germination rate (92%), and lower standard deviation (± 3.5) in total germination compared to the other species (91 ± 8.1%, 94 ± 8.1%, and 86 ± 8.3% for radish, lettuce, and turnip, respectively).

Determination of Triclopyr Concentration in the Field Using a Bioassay and Comparison with a Laboratory

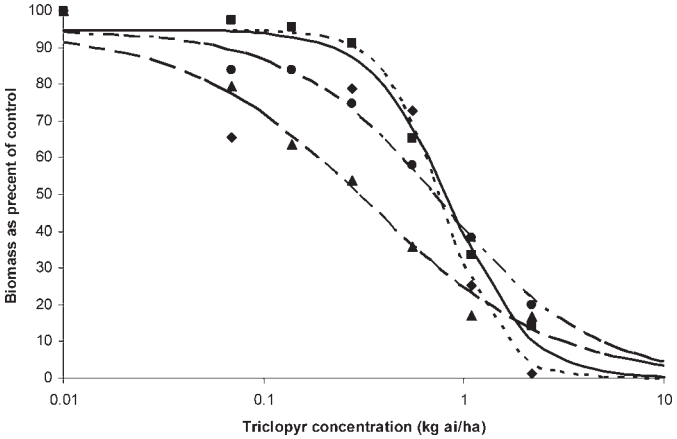

Figure 1. Dose–response curves of radish (solid line and square), turnip (long dash line and triangle), lettuce (short dash line and diamond), and mustard (long and short dash line and circle) to 1 wk of growth in triclopyr-treated soil. Points represent means of response. The no herbicide control is represented as 0.01 kg ai ha^{-1}. Equations for dose–response: radish $f = 0.01 + (94.76 - 0.01)/\{1 + \exp(5.01) \times [\log(x) - \log(0.85)]\}$; turnip $f = 0.01 + (94.76 - 0.01)/\{1 + \exp(2.19) \times [\log(x) - \log(0.33)]\}$; lettuce $f = 0.01 + (94.76 + 0.01)/\{1 + \exp(6.7) \times [\log(x) - \log(0.78)]\}$; and mustard $f = 0.01 + (94.76 - 0.01)/\{1 + \exp(2.69) \times [\log(x) - \log(0.78)]\}$.

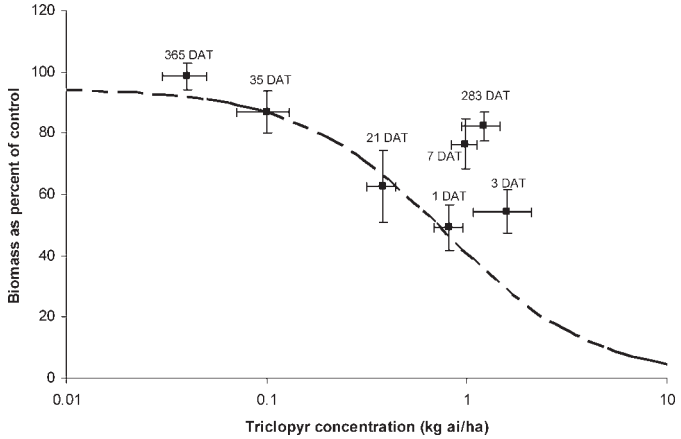

Figure 2. Dose–response curves of mustard after 1 wk (dashed line) of growth in triclopyr-treated soil and comparison of soil bioassay and chemical extraction methods for determining soil concentration of triclopyr over time in a field study. The no herbicide control is represented as 0.01 kg ai ha^{-1}. Equation for 1 wk dose–response bioassay is $f = 0.01 + (94.76 - 0.01)/\{1 + \exp(2.69) \times [\log(x) - \log(0.78)]\}$. Points are the (X, Y) coordinates for chemically extracted triclopyr concentration and bioassay determined mustard biomass, respectively that were determined from field samples taken 1, 3, 7, 21, 35, 283, and 365 d after treatment with 2.2 kg ai ha^{-1} triclopyr in Delta Junction, AK. Horizontal bars are the standard error of the chemical extraction method and vertical bars are the standard error of the lab bioassay.

Extraction. At 1 DAT, the bioassay resulted in 49 ± 7% SE of the biomass of the control plants and the laboratory extraction methods determined a triclopyr amount of 0.82 ± 0.14 SE kg ai ha^{-1} (Figure 2). These values when plotted with the 1-wk dose–response bioassay developed with known amounts of triclopyr indicate that the bioassay and the chemical extraction are equivalent. Given the vegetation coverage at these sites, it is reasonable to assume that about half the applied herbicide was not intercepted by plant material and had reached the soil surface. At 3 DAT, the extracted herbicide concentration in the soil increased but phytotoxicity had not changed (Figure 2). Other than morning dew, there had been no precipitation events to wash additional herbicide off the plant material (Figure 3). At 7 DAT the phytotoxicity and extracted amount of triclopyr in the soil had declined; however, the chemical extraction was again overestimating triclopyr in the soil compared to the bioassay results. The reduction in toxicity can be due to leaching below the sampled depth and microbial degradation of triclopyr in the soil (Lee et al. 1986). With the reported half-life for triclopyr of 10 to 100 d (Cox 2000), the loss of phytotoxicity was expected. At 21 DAT, triclopyr concentration had declined and phytotoxicity was again at the expected range based on the 1-wk dose–response bioassay. Between the 7 DAT and 21 DAT sampling 1.03 cm of precipitation was measured (0.18, 0.03, 0.79, and 0.03 cm on 9, 10, 13, and 20 DAT, respectively). This precipitation may well have washed active triclopyr off previously mowed vegetation and the surfaces of live vegetation, resulting in a slightly increased level of phytotoxicity such that extracted triclopyr was again at levels predicted by the bioassay. This movement of triclopyr described above was observed in two other studies (Stephenson et al. 1990; Thompson et al. 2000). It is possible that the wet soil may have made triclopyr more available for plant extraction. At 35 DAT, the amount and phytotoxicity of triclopyr had declined significantly and both methods were estimating similar amounts of triclopyr in the soil. Although an additional 2.8 cm of precipitation had occurred between the 21 and 35 DAT sampling dates, there were no further additions of triclopyr to the soil. At 35 DAT extracted triclopyr concentration was equivalent to 0.1 kg ai ha^{-1}, an eighth of what was measured during the first sampling event.

At 283 DAT (April 27, 2007), when soils had thawed, the extracted triclopyr concentration (1.21 kg ai ha^{-1}) was similar to concentrations measured the first week after treatment; however, phytotoxicity as measured in the 1 wk mustard bioassay determined only an 18 ± 5% SE decrease in growth compared to controls (Figure 2), which would be equivalent to 0.04 kg ai ha^{-1} triclopyr. The results of the bioassay at 238

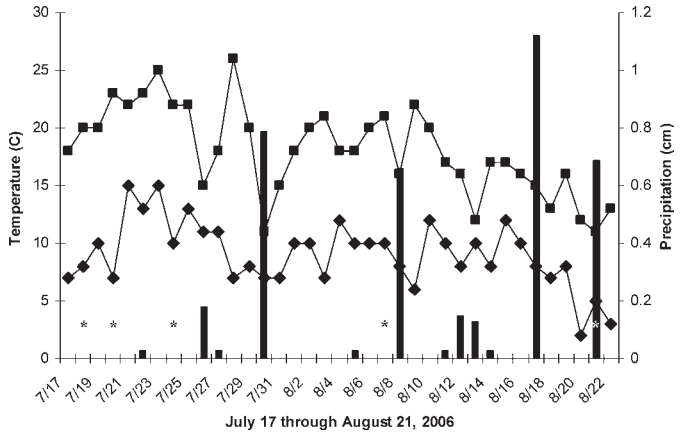

Figure 3. Daily high (boxes) and low (diamonds) temperature and precipitation (bars) during the first 35 d of soil sampling near Delta Junction, AK. Treatments were applied on July 17 and sample dates are marked with an asterisk.

DAT were no different than the bioassay results at 35 DAT. During winter we hypothesized that frozen soils would inhibit degradation of triclopyr and that at 283 DAT we would measure something less than 0.1 kg ai ha^{-1} triclopyr. Research conducted by Lewer and Owen (1990), determined that the susceptible plant chickweed (*Stellaria media* L.) did not metabolize triclopyr as rapidly as more tolerant plants (barley [*Hordeum vulgare* L.] and wheat [*Triticum aestivum* L.]). They also determined that the major metabolite in the susceptible plant was triclopyr aspartate, which can be hydrolyzed to the phytotoxic triclopyr acid. It is possible that at spring thaw triclopyr and triclopyr aspartate could have been released from triclopyr-susceptible and other vegetation that had been killed the previous autumn or during the first hard freeze in mid-September, about 55 DAT. Several studies (Lee et al. 1986; Norris et al. 1987; Jotcham et al. 1989) have all detected triclopyr and triclopyr metabolites in the surface layer of soils months after application, using chemical extraction techniques. In a study by Jotcham et al. (1989), triclopyr 2 mo after application, was found to be no longer toxic to lentils (*Lens culinaris* Medik.) as a bioassay plant, even though it was still present in the soil. Therefore, we conclude that the extraction process was resulting in a false positive and possibly overestimating the concentration of triclopyr in the spring 283 DAT. At 365 DAT triclopyr concentrations were close to the limit of detection and no phytotoxicity was measured (Figure 2).

To determine the half-life of triclopyr, data were used for the first 35 d of the study when triclopyr degradation was most active. The extraction and bioassay data resulted in correlation coefficients of −0.96 (R^2 equal to 0.91) and −0.80 (R^2 equal to 0.64), respectively, using first-order decay kinetics (data not shown). These results determined a calculated 10-d half-life for the chemical extraction method compared to a 22-d half-life for the bioassay. Limiting the calculation to this time period makes sense owing to the approximately 6-mo period that the soil was frozen between approximately day 35 and day 283 of the study. During this period little to no degradation of triclopyr occurred as indicated by the bioassay results (Figure 2). Thus, the calculated half-life is just for the time period that the soil is thawed. These rates of degradation would be comparable to rates measured at lower latitudes (Cox 2000) and similar to those measured in forest soils near Fairbanks, AK (Newton et al. 2008).

Separately, the methods of chemical extraction and bioassay only convey part of triclopyr's activity in the soil. The chemical extraction measures the concentration of triclopyr, but not the dose that may affect the crop. The bioassay measures the concentration of triclopyr that is available to the plant, but not unavailable active herbicide in the soil. The dose–response curve predicts when there may be an adverse effect on plant biomass at a certain concentration of triclopyr and when there may be no adverse effect. In analyzing these methods together, we gain a more complete picture of what the concentration of herbicide was and what the impact on mustard biomass was at different points in time. With this information, we may better understand the fate of triclopyr in the soil and predict the potential for carryover injury of triclopyr susceptible plant species.

This study has application for agriculture, invasive weed control, and cold climate research. In agriculture and invasive weed control, the importance of degradation rates of applied herbicides and how they may affect a sensitive rotational crop or planted restoration species in the future is critical to making good management decisions. In cold climate research, it is important to understand how a herbicide will degrade and move in the soil during the summer months as well as where the herbicide may reside during the winter months. Future research is needed to determine the cause of increased triclopyr concentrations found in the spring soil 283 DAT. This information could be important for herbicide-sensitive rotational crops and other agricultural practices in interior Alaska. Similar research will need to be conducted on other herbicides and crops for a better understanding of the impact soils and climate in the interior of Alaska have on herbicide movement and fate.

Sources of Materials

[1] Plastic tray w/clear lid, LOWE'S of Fairbanks, AK #1985, 425 Merhar Ave., Fairbanks, AK 99701.

[2] Triclopyr, The Dow Chemical Company, 2030 Dow Center, Midland, MI 48674.

[3] Seeds, Osborne International Seed Company, 2428 Old Hwy. 99 South Rd., Mount Vernon, WA 98273.

[4] TruSpec, Leco Corporation, 3000 Lakeview Ave., St. Joseph, MI 49085, www.leco.com.

[5] Corning 220 pH meter, American Instrument Exchange, 1023 Western Ave., Haverhill, MA 01832, http://www.americaninstrument.com/index.asp.

[6] CO_2 backpack sprayer, R & D Sprayers (Bellspray Inc.), 419 Hwy. 104, Opelousas, LA 70570, http://www.co2sprayers.com.

[7] Teeject 8002 even flat-fan spray nozzle. Spraying Systems Co., Wheaton, IL 60187.

[8] Petri dishes, VWR International, 1310 Goshen Parkway, West Chester, PA 19380, http://www.vwrsp.com/index.cgi.

[9] Growth chamber, Conviron 2005, model PGR15, Controlled Environments Limited, made in Canada, http://www.convirion.com.

[10] Turbovap II, Zymark Corporation, Sotax Corp., 411 Caredean Dr., Ste. A, Horsham, PA 19044.

[11] Agilent 6890N Network Gas Chromatograph system with a 5973 Network Mass Selective Detector, Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051, http://www.agilent.con/chem.

[12] SAS software, Version 9.1, SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513.

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